Manuscript

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Peroxidation of Lipids and Oxidative Modification of Proteins in Synovial Fluid of the Knee Joint under Normal Conditions and Degenerative Dystrophic Changes

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Abstract

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Dissertation Council
Academic Secretary
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GENERAL DESCRIPTION OF RESEARCH

Relevance. Deforming osteoarthritis (DOA) of large joints is typically the worst case; it tends to rapidly bring about permanent disability and to significantly impoverish well-being (Anderson D.D.et al., 2011). Intensifying peroxidation processes proves to be quite influential in affecting etiology and pathogenesis of various diseases and manifestation of adverse aftermath. As the pathology progresses, the balance between the formation of peroxides and their consumption together with other products is likely to be upset. As a result metabolites tend to accumulate in tissues and biological fluids, this leading to severe morphological changes. Owing to the impact of peroxidation on the pathogenesis of various diseases, determining process products in biological material, namely, blood serum and synovial fluid (SF) is of increasing diagnostic and prognostic significance. In traumatology and orthopedics features of the system of lipid peroxidation (LPO) in the serum of the patients suffering from OA in their hips have been carefully examined, which paved the way to developing the algorithm for predicting and diagnosing unstable endoprostheses (Istomin S.U. 2008).

However, in modern literature there is hardly any information regarding research into lipid peroxidation processes and oxidative modification of proteins (OMP) with degenerative changes in the joints (DDSJ) related to synovial fluid. Thus way, focusing on synovial fluid and developing prognostic criteria for examining imperfections in the structure of the extracellular matrix and articular and para-articular tissues to cover the entire progress of degeneration in the joint is topical and promissory, the need for putting it in practice is beyond any doubt.

The purpose of research is an in-depth study of fluctuating parameters pertinent to lipid peroxidation and oxidative modifications of proteins in synovial fluid as the joint continues to degenerate. The study is correlated with the stages of the process and its etiology.

Objectives:

1. Identifying indicators for protein and lipid profiles, lipid peroxidation and OMP in synovial fluid and serum by examining experimental animals under normal conditions and by simulating early and later stages of degenerative lesions in the joint.
2. Determining the performance of peroxidation in the synovial fluid of large joints under normal conditions making use of resective material. Analyzing comparatively the processes of peroxidation of synovial fluid in large joints (for example, synovial fluid of the knee and elbow joint).
3. Studying changes in LPO and OMP in the synovial fluid and serum of patients with degenerative-dystrophic lesions of large joints in the early and late stages (idiopathic gonartrozI-II stage, idiopathic gonartrozIII stage).
4. Performing a comparative analysis of the synovial fluid peroxidation in patients with osteoarthritis of various etiologies (idiopathic, post-traumatic, dysplastic).

5. Determining feasible criteria for the prognosis of unstable components in the endoprosthesis following primary knee arthroplasty.

Novelties:

Reference values have been determined for peroxidation, lipid and protein metabolism, activity of catalase in normal SF large joints. Comparative analysis revealed that the biochemical composition of SF healthy knee and elbow joints varies regarding human total protein concentrations, total lipids, cholesterol, triglycerides and concentrations of MBP – ketone secondary products.

New data have resulted from studying changes in the processes of lipid peroxidation and protein SF knee correlated to the progress of LLINs development, experimental and clinical material being applied for this purpose. Accumulation of lipid peroxidation products has proved to occur with a predominance of primary metabolites, i.e. aldehydes and diene conjugates, for the cases of LLINs traumatic, dysplastic and idiopathic etiology.

Differences between the values obtained in laboratory tests on patients with stable and unstable endoprosthesis have been identified. These findings substantiate the importance of examining the products of lipid peroxidation and catalase activity in synovial fluid in order to predict the instability of the knee joint when primary arthroplasty is performed.

Theoretical and practical significance:

The results justify that studying parameters of lipid peroxidation and oxidative modification of proteins in synovial fluid in the clinical diagnosis of degenerative joint lesions to assess the severity of pathology is of paramount importance. Definition of lipid peroxidation products and antioxidant enzyme catalase activity can improve objective estimation of the joint environment, and it is certain to facilitate risks of instability after primary arthroplasty of the knee has been done. Additionally, basic theoretical principles of the research can be used in the lecture courses "The Clinical Application of the Study of Synovial Fluid" for postgraduate students and specialists specializing in orthopedics and traumatology.

Methodology and Research Methods:

The present research was done in the biochemistry laboratory of the clinical and experimental department at the Ilizarov Research Centre operating under the Russian Ministry of Health. An approval was granted from the Ethics Committee at the Ilizarov Research Centre. Keeping animals, surgery and euthanasia have been implemented in compliance with the humane attitude to the objects of study and all ethical norms have been observed as stipulated in the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" and the Decree of the Health Ministry of the Russian Federation, 23.08.2010, # 708n "On Approval of Rules of Laboratory Practice" (Registered in the Ministry of Justice 13.10.2010 N 18713). Experimental animals were provided with separate enclosures and a balanced diet.

Biochemical Methods:

Total protein (TP) was determined by the biuret method applying VitalDiagnostic sets, electrophoretic separation of protein fractions was performed without pre-treatment synovial using a Helena equipment, «BioSciensEurope», England; albumin-globulin ratio, the proportion of α-, β- and gamma-globulins were computed at the same time. OMP products were measured in a protein pellet making use of the 2,4-dinitrophenylhydrazine reaction. Primary products, aldehydes, were detected at wavelengths of 270 nm, 363 nm and 370 nm (OMP270), while secondary ketones (OMP363 + 370) were done at 370 nm (OMP270). Concentration of OMP products were calculated in absorbance units per mg protein (Vyushin A.V.2002.). LPO Intermediate substances were applied such as diene conjugates (DK) and malonic dialdegid (MDA) to serve as quantative markers for the depth and extent of pathology. DK content was examined with the help of spectrophotometry looking for the differences in optical density between experimental and reference samples at 232 nm wavelength (Orekhovich V.N. 1977). Thiobarbituric acid was used to determine MDA. Concentrations of peroxidation products was calculated per mg of general lipids (GL), which in turn were discovered Lachema sets (Czech Republic). Holesterol concentrations (HL), and triglycerides (TG) were obtained by the colorimetric method based on determining substance concentration of the color intensity of the solution (Vital Diagnostic sets). Activity of enzyme catalase was examined by spectrophotometry at a wavelength of 410 nm, revealing the ability to form a stable colored complex of hydrogen peroxide with molybdenum salts (Koroljuk MA 1988). The results of lipid peroxidation and protein oxidation were presented as a sum of the calculated coefficient [DK + MDA], [Aldehydes +Ketones] and relations [DK / MDA], [Aldehydes / Ketones].

Statistics:

Statistical processing the data was performed by variational statistics, which is used for small-scale sampling. Experimental and clinical research was based on representative sampling excluding the distribution popup options and checking for normal distribution. The study included mathematical planning. Median values and interquartile swings of 0.25 and 0.75 percentile were calculated for the observation group. Glantz's statistical methods, algorithms and methods, calculation formulas were applied (Glantz S. 2008).

A factor of the linear correlation of Pearson (r) has been calculated. Differences in the two groups were verified with the help of Wilcoxon nonparametric test, taking advantage of licensed software (Gaydyshev I.P. 2004). Differences were considered significant at $p < 0.05$

Propositions:

1. The biochemical composition of the synovial fluid in the knee and elbow joints in the human norm varies in terms of the total protein and general lipids, lipid and protein oxidative modification.

2. As degenerative changes in the synovial joint are going on, accumulation of lipoperoxidation products and redistribution of products resulting from oxidative modification become evident fluid revealed; it depends on the severity of the pathological process, while the etiology is irrelevant.

3. Parameters of lipid peroxidation and antioxidant enzyme catalase activity can be used as criteria of forecasting instability in patients' endoprostheses after primary knee arthroplasty.

Verification and Approbation:

The research has been done based on a sufficient amount of factual material relying on up-to-date biochemical methods.

The thesis was presented at the following conferences: "Laboratory Science - Practice: the First Decade of the XXI Century" (Moscow 2009); "Technology and Innovation in Laboratory Medicine" (Moscow 2009); «VIII Congress of the Russian Arthroscopic Society" (Moscow 2009); "IX All-Russian Congress of Orthopaedic Trauma, Dedicated to the 200th anniversary of Pirogov" (Saratov 2009); "Topical Problems of Theoretical and Applied Biochemistry" (Chelyabinsk, 2009); "Issues of the Upper Limb Surgery" (Kurgan 2009); "The Session of the Regional Society of Orthopaedic Trauma" (Mound, 2009), (Kurgan 2013); "Osteoporosis and osteoarthritis - the Problem of the XXI Century" (Kurgan 2009); "Ilizarovskie Chteniya" (Kurgan 2010).

Personal contribution:

The author performed a comprehensive survey of the information on the subject. The data and context presented in the thesis have been compiled, processed, analyzed and interpreted solely by the author. She fully applied the techniques for studying biochemical parameters in 384 patients, and 30 mongrel dogs and directly involved in collection of materials for research, biochemical analysis and processing together with computer data processing and statistical analysis. The applicant's participation in preparing and writing articles was overwhelming.

Publications:

The subject of the research was highlighted in 16 papers, 7 of them were published in the journals recommended by the Higher Attestation Commission of the Russian Federation for the Publication of the Results of the Research.

Volume and structure:

The thesis consists of an introduction, five chapters, conclusions, list of references, including 238 sources (of which 179 are domestic, 59 coming from foreign publications); it contains 121 pages of typewritten text and is illustrated with 7 figures and 24 tables.

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The thesis work is done as part of the theme of research state registration number 036 / 2-19 of the Federal State Organization "Russian Scientific Center" Restorative Traumatology and Orthopedics "named after academician GA Ilizarov Russian Ministry of Health, " Kurgan.

HIGHLIGHTS OF WORK

Experimental modeling of osteoarthritis (OA) were carried out on adult mongrel dogs. The basic material of the study was the synovial fluid of the knee and the blood serum of 30 mongrel dogs: intact (n = 10), with a model of degenerative lesions of the joints (n = 20) in the early stages (28 days of the experiment, n = 14) and with the model of degenerative -distroficheskih affected joints in the later stages of the disease (56 days of the experiment, n = 6). The used experimental model implies compression of the articular surfaces of the knee joint with the creation of an ischemic component by the intersection of the femoral artery (RF Patent 2452999, IPC G 09B23 / 28 simulation method of osteoarthritis of the knee / Makushin VD Stepanov MA, TA Stupina №2011104885 / 14, stating 09.02.2011, Opubl.10.06.2012, Bulletin, №16).... The criterion for timing of the experiment served as a clinical and radiological characterization of the state of joints. Knee dog joints and man are similar in anatomy, the regeneration of cartilage and type of location that is considered important in the extrapolation of experimental data (Derevyanko IV 2004). Consequently, the selection of animals and it is possible to simulate the most convenient degenerative changes. According to the classification of the main periods in the development of degenerative changes in the joints of the initial changes in the joints of experimental animals correspond to the earliest period of development of the pathological process in humans (Makushin VD et al., 2005.).

Proceeding from the objectives of the study clinical material was distributed to the following groups:

I clinical group consisted of material from 45 patients with idiopathic gonarthrosis of the knee joint stage I-II: men - 27 and women - 18, the average age was 69,3 ± 2,6 years.

II clinical group consisted of material from 293 patients with idiopathic gonartrozomIII stage: men - 78 and women - 215, mean age 73,0 ± 2,3 years.

In the III clinical group has been studied the composition of synovial fluid from 30 patients with damage to the meniscus or cruciate ligament. The patient group consisted of: men - 19 and women - 11, mean age was 61,3 ± 2,1 years.

In the IV clinical group presented material synovial fluid of 16 patients with osteoarthritis of the knee dysplastic etiology. This group included: men - and women 4 - 12, the average age of 57,2 ± 2,2 years.

V clinical group consisted of material from 217 patients from among the patients, who were operated on for primary knee arthroplasty. Of these, 208 patients with stabilnymendoprotezom: men - 96 and women - 112, the average age of 71,4 ± 2,8 and 9 patients with instability of the endoprosthesis: men - women and 2 - 7, the average vozrast67,4 ± 3,1 years. A group of patients with unstable endoprosthesis has been allocated during the period of our study, namely, within 3 years.

The control group consisted of 45 samples of material from the cutting 30 corpses suddenly dead people of both sexes: men - 19 and women - 11, the average age of 55,3 ± 1,6 years (30 samples of synovial fluid of the knee joint and the elbow joint 15 samples).

In addition, serum samples of 50 patients with degenerative-dystrophic changes were investigated: men - 21 and women - 29, average age sostavil59,3 ± 2,6 years. The analysis of clinical and diagnostic laboratory to laboratory control of the relevant joint pathology was examined sera of patients with this gruppy.Takzhe results of serum samples of 10 healthy volunteers were obtained: men - izhenschin 4 - 6, the average age of 58,5 ± 1,8 years. They were determined biochemical parameters of lipid peroxidation and protein.

All patients in the study were given informative voluntary consent to medical intervention and publication of data resulting from the study.

RESULTS

Results of the study parameters peroxidation in synovial fluid and serum in animal model of degenerative changes in the knee joint.

Based on the results of studies in experimental animals SF we noted that in the early stages of the degenerative process in a joint concentration GL and HL remained at the preoperative level, but there are changes in the lipid spectrum of the synovium - a statistically significant increase in the number of TG. Concentration DK peroxidation product increases with respect to normal values by 2 times, and the concentration of the secondary product MDA, reduced 3-fold relative to the norm. A key activity of antioxidant enzymes - catalase - remained within the normal range. Estimated sum peroxidation products [DK + MDA] went down, because It has lowered the concentration of secondary lipid peroxidation products - MDA. The calculation of the ratio of primary and secondary lipid peroxidation products [DK / MDA] showed it to increase, indicating that their redistribution to the decrease in the proportion of secondary LPO products - MDA and increase the content of primary products - DK.

The concentration of total lipid in the simulation SF OA at later stages of the disease was significantly improved. In terms of the lipid spectrum above normal values observed indicators HL and

triglyceride concentrations. DK concentration rose 3 times, and MDA concentration decreased 2-fold relative to normal levels. Catalase activity remained virtually unchanged. Calculation of the coefficients [DK + MDA] and ratio [DK / MDA] primary and secondary lipid peroxidation products showed them to increase, indicating that the accumulation of lipid peroxidation products and the prevalence of primary products DK (Figure 1).

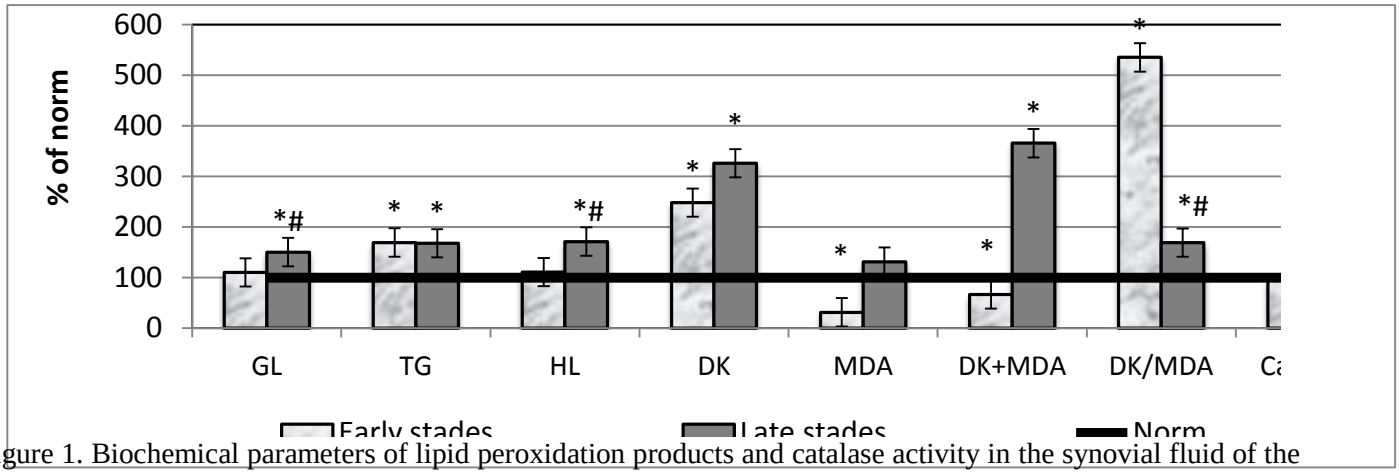


Figure 1. Biochemical parameters of lipid peroxidation products and catalase activity in the synovial fluid of the knee OA dogs in modeling in the early and late stages of DDCJ

Hereafter * - the difference is statistically significant with a significance level r≤0,05otnositelno rules Hereinafter # - differences are statistically significant with a significance level of $p \leq 0.05$ between groups

With the development DDJC of TP concentration increases significantly. The amount of primary protein peroxidation products in the early stages of the experiment is reduced in terms of the absolute values of aldehydes. Also significantly reduced the concentration of ketones. The amount of OMP products [Aldehydes Ketones +] was significantly reduced relative to the norm, and their ratio [Aldehydes / Ketones] showed that the rate of aldehydes decreased to a much greater extent.

In the later stages of the disease in SF is a significant accumulation of lipid peroxidation products. Obviously, oxidative stress manifests itself in a wide range of free radical generation of LPO products. Investigation of protein at early stages of the spectrum showed no differences from the normal values, and in the later stages showed a statistically significant reduction in the concentration fractions of α1-globulin, α2-globulin and β-globulin fraction. A significant reduction in the concentrations of these fractions is offset by an increase in the concentration of γ-globulin fraction that characterized the process as chronic. In the later stages of the experiment concentration TP significantly increased, and the concentration of OMP products also had a statistically significant decrease in performance (Figure 2).

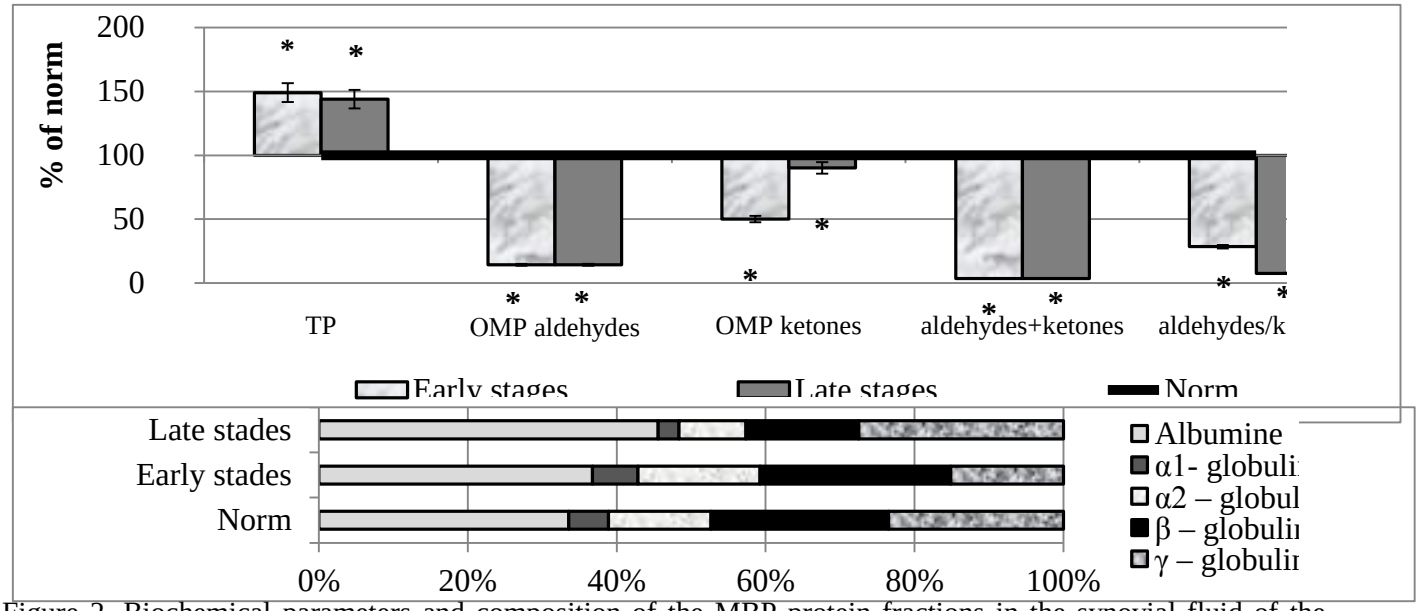


Figure 2. Biochemical parameters and composition of the MBP protein fractions in the synovial fluid of the knee OA dogs in the simulation at the early and late stages DDISPrimechanie - See Fig. 1

Biochemical studies of blood serum in the early stages of the disease showed a statistically significant increase in the concentration of lipid peroxidation products, and accordingly, the excess of the total concentration of lipid peroxidation products [DK + MDA], but 1.5-fold decrease in OMP products unbalance indicating lipid peroxidation products and proteins the accumulation of free radical lipid compounds (Figure 3).

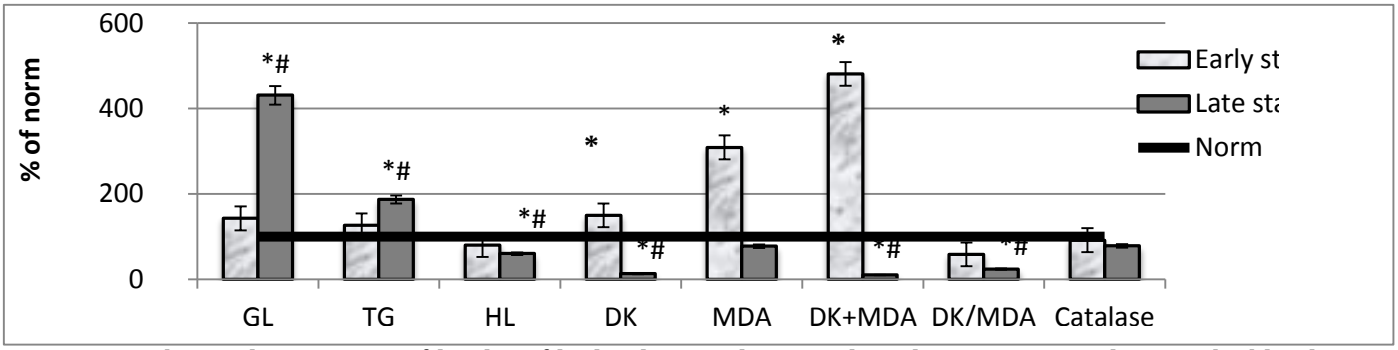


Figure 3. Biochemical parameters of lipid profile, lipid peroxidation and catalase activity products in the blood serum of dogs with OA of the simulation in the early and late stages DDISPrimechanie - See Fig. 1

In the late stages of OA changes in the biochemical composition of blood serum were more pronounced: 3.5-fold increased concentration of the GL, 2 times the concentration of triglycerides. There was a significant (8-fold) reduction in the concentration of DK, and some (23%) decrease MDA content, which we have not seen in the early stages of OA development. The activity of the main antioxidant enzymes - catalase remained unchanged.

It is noted that in dogs at different stages of joint pathology studied serum indicators differed from the control group and have the features (Figure 4).

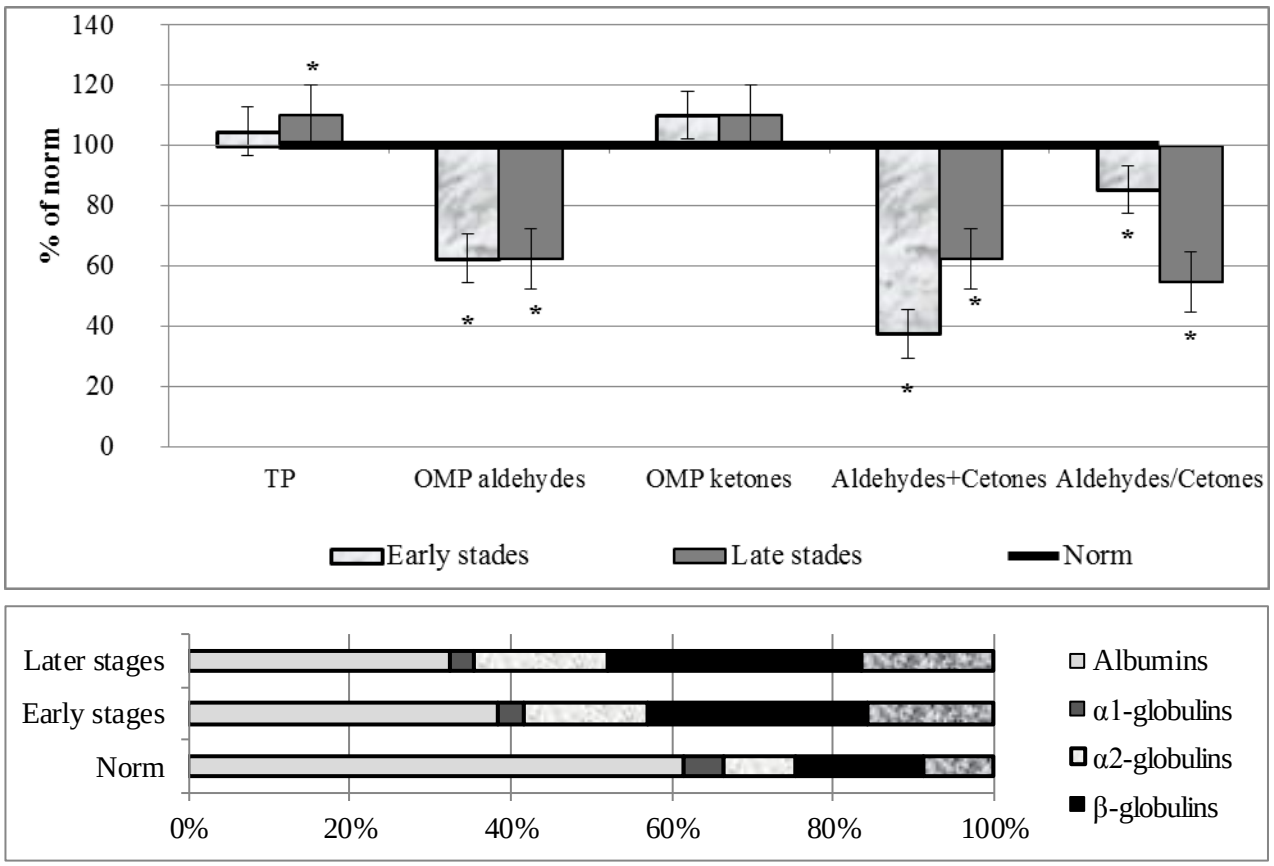


Figure 4. Biochemical parameters total protein, MBP products and composition of protein fractions of blood serum of dogs with OA of the simulation in the early and late stages of DDCJ Note - See Fig. 1

The concentration of primary products OMP - aldehydes statistically significantly decreased the level of significance of $p < 0.05$. It is known that the product forms Schiff bases with amino groups of proteins, speaking at the same "cross-linking" agent. As a result of "cross-linking" form insoluble "complexes pigments' wear, or end-products of oxidation within the cell proteins. However, the content of secondary products of protein peroxidation - ketones - was at the preoperative level. Having considered the settlement koeffitsientysummy [Aldehydes Ketones +] and ratio [Aldehydes / Ketones], it should be noted their decline.

Studies conducted by us in blood serum of the dogs in the later stages showed that TP concentration was higher than the control values. The concentration of OMP products significantly decreased only in primary products - aldehydes, the concentration of secondary products - ketones - remained unchanged. Accordingly, in view of reducing the concentration of OMP primary products have been reduced, and the estimated coefficient [Aldehydes Ketones +], and the ratio of [Aldehydes / Ketones].

In the early stages of development of DDCJ in serum of dogs observed d-dystrophic ysproteinemia. Reduced albumin may be due to some loss of protein that can be caused by acute inflammation, namely about said raising α2-globulin fraction. Increased γ-globulin fraction directly indicates the development of DDCJ. With the development of DDCJ was significantly reduced

concentration α1-globulin fraction. In the later stages of the process, it was reduced to a greater extent. In the later stages of an increase in α2-globulin, β-globulin and γ-globulin fractions indicates the presence of inflammation and chronic forms of the disease. It should be noted that changes in lipid and protein spectra peroxidation products in concentrations of SF in the development of pathological processes occur earlier than in blood serum.

Research results indicators peroxidation in synovial fluid and serum of patients with degenerative-dystrophic changes in joints.

To conduct a comparative analysis of biochemical parameters of the composition SF was necessary to study the synovium samples of people without joint pathology. Composition SF other joints besides the knee, has not been investigated, and in the available literature, we found no such information. Based on this study, it was found that SF knee and elbow joints is observed complete identity of the biochemical composition (Figure 5).

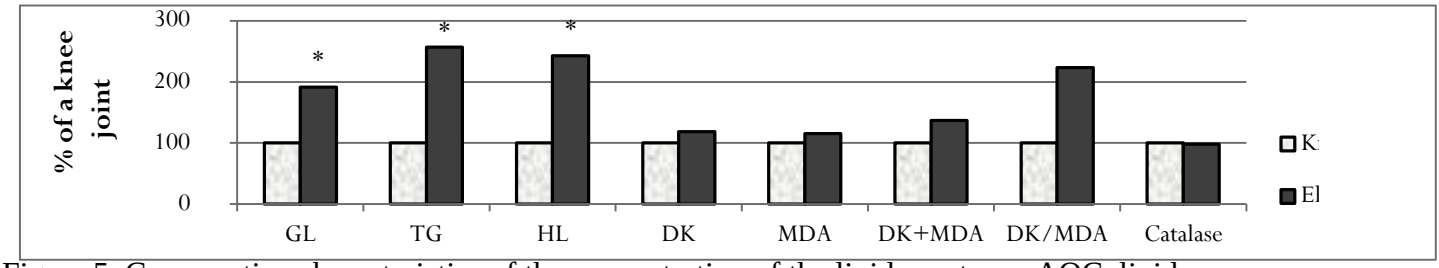
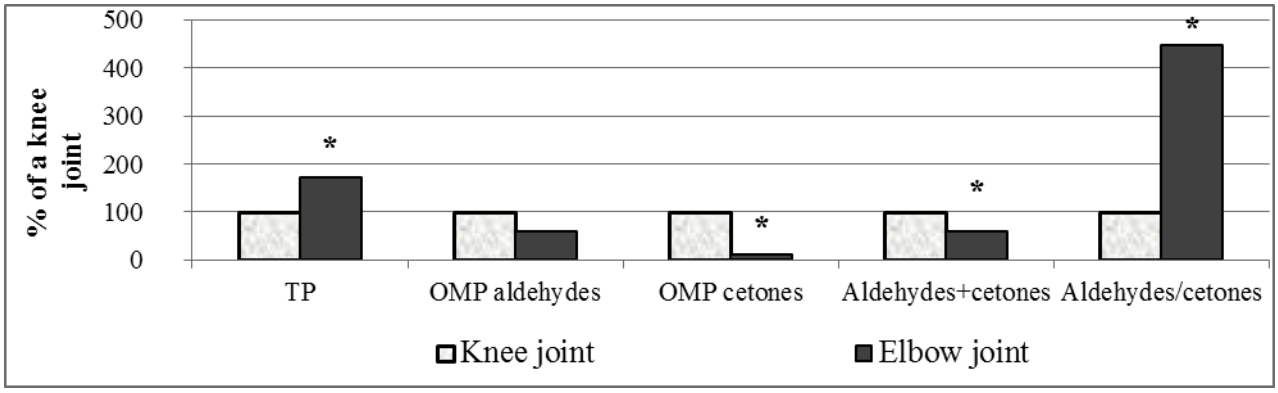


Figure 5. Comparative characteristics of the concentration of the lipid spectrum, AOC, lipid peroxidation products in the synovial fluid of the knee and elbow joints in normal human

Concentration GL of the elbow joint was significantly higher than those in the knees. It was also noted that the concentration of lipid metabolism (HL, TG) was lower than the knee. LPO Indicators in the knee and the elbow did not have any differences that can be said about the estimated coefficient [DK + MDA] and ratio [DK / MDA]. The activity of antioxidant enzymes - catalase - was on the same level.

Thus, it was found statistically significant differences in the concentrations HL, TG, GL LPO products - all concentrations significantly decreased in the knee joint.

The products OMP differences between knee and elbow joints were only secondary products OMP - ketones, which were statistically significant increase in knee joint (Figure 6).



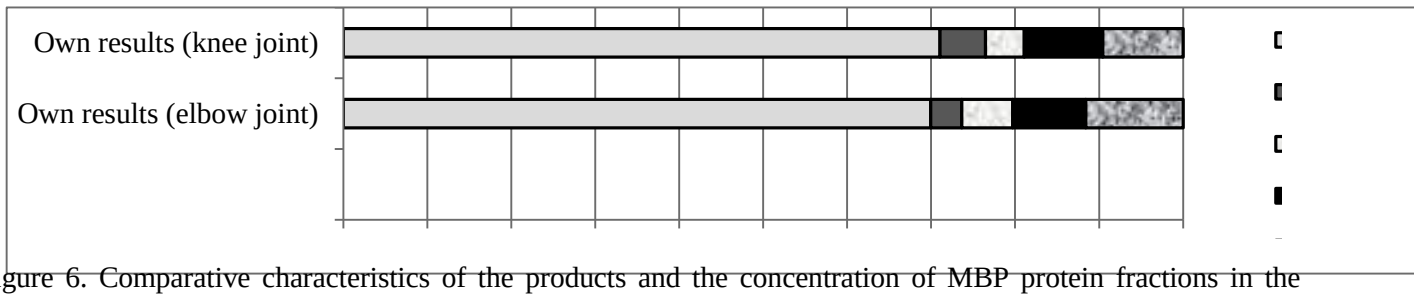


Figure 6. Comparative characteristics of the products and the concentration of MBP protein fractions in the synovial fluid of the knee and elbow joints in normal human

From 6 the data presented in the figure can be seen that there are some differences in the composition of SF different human joints. There were no statistically significant differences in the values of the albumin-globulin ratio, however, they were in a content of protein fractions.

When determining the reference values of the biochemical composition of SF, obviously, should be considered a strict relationship between the shape, size, movements, nature and content of the joint environment, which is due to varying degrees of joint load. Lubricating properties of the medium across the hinge devices should have different physico-chemical characteristics and therefore different biochemical composition.

To accomplish the objectives set in our work, we investigated similar biochemical indicators of SF and serum on the clinical material of patients with different etiology of the disease and at different stages of development of the pathological process.

In the diagnosis of diseases of the joints SF research is essential. Synovial fluid is the major organ specific components of each joint. It is responsible for physical and chemical properties and biochemical composition responds to any intraarticular processes. The efficiency of both surgical and conservative treatment of degenerative diseases of the joints shows that the method of therapy depends not only on the chosen method of treatment, but also on the compensatory capacity of the organism.

With the development of idiopathic arthrosis (IA) I-II stage of SF patients, we found an increase in the concentration of 2 times AL, 2.5 times - HL and decrease in TG concentration of 5 times. The same pattern we found in patients with SF IA III stage. The concentration of lipid peroxidation products was statistically significant higher than the norm, as in the earlier and the later stages of the IA, mainly due to the accumulation of the primary products of lipid peroxidation. Catalase activity remained unchanged (Figure 7).

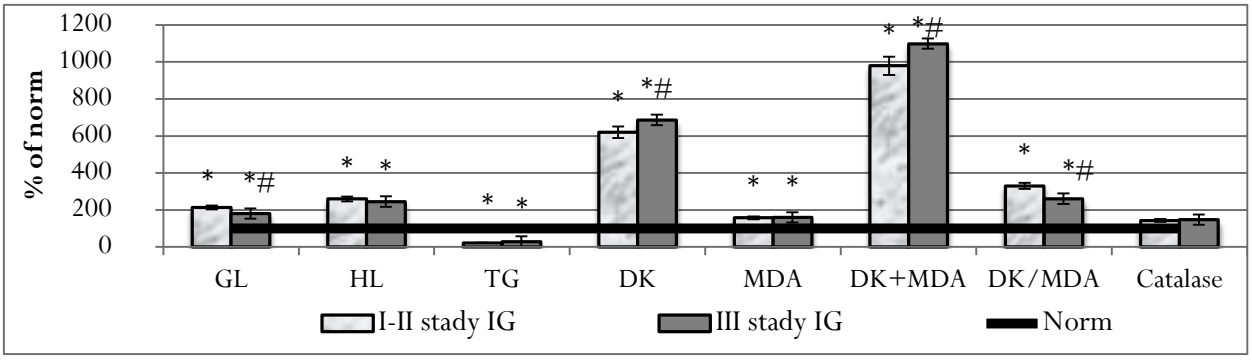


Figure 7. Biochemical parameters of lipid metabolism and lipid peroxidation-AOC system in the development of idiopathic gonarthrosis (IG) in the early and late stadiyahPrimechanie - See Fig.. 1

Increasing the content of the statement in SF observed in the vast majority of diseases that occur with the phenomenon of synovitis (Figure 8). During the OMP in the development of the disease from early to late stages of the accumulation of aldehydes: 2 times on stage I-II and 4 times at stage III disease. Secondary products OMP early and late stages of falling in 2.5 times. However, in the process step III SF accumulation OMP products by increasing the concentration of aldehydes. Redistribution of protein fractions of SF patients were completely identical in both the early and the later stages of the IA. It is expressed in the decrease in α-globulin fractions and fractions increase β-globulins. Our results indicate a pronounced signs of oxidative damage to lipids and proteins in the development of SF when IA.

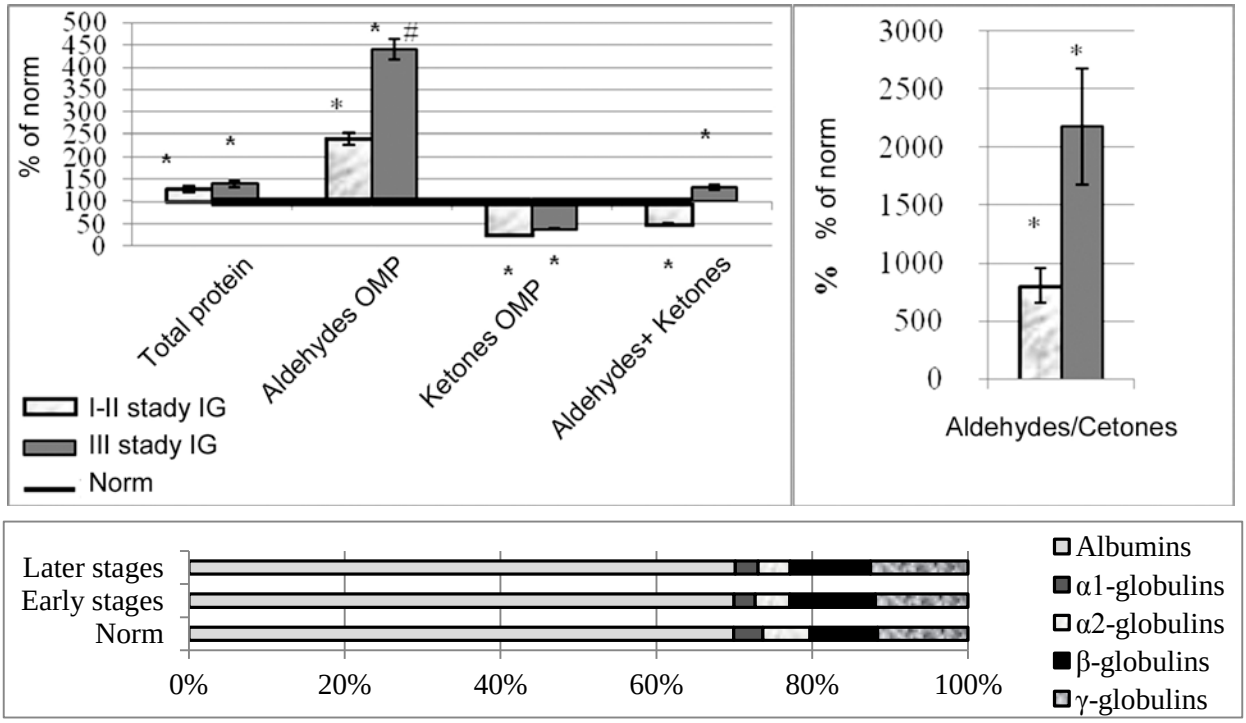


Figure 8. Biochemical parameters total protein products OMB and composition of protein fractions in the development of idiopathic gonarthrosis (IG) in the early and late stadiyahPrimechanie - See Fig. 1

Analyzing the indicators of oxidative status, note that the concentration of DK and MDA reached the maximum values in excess of control several times in the later stages of the disease. The accumulation of lipid

peroxidation products and OMP in the synovial fluid of patients with idiopathic gonarthrosis at different stages of the disease is the result of free radical oxidation of lipids and proteins, and occurs simultaneously with razbalansirovkoysistem regulating the intensity of peroxidation. Evaluation of free radical homeostasis SJ has demonstrated the equilibrium shift towards the accumulation of the primary products of active oxygen metabolites.

According to our data in blood serum of patients osteoarthrosis different stages of the disease are changes of biochemical parameters of lipid obmena.Nesmotrya that concentration GL in the blood serum in patients with knee OA at all three stages of the disease remains in the normal range, there are variations in the level of concentration of HL and TG. All three stages of the disease, these figures are increasing consistently, differing from normal with different levels of importance. The concentration of HL 1.4 higher in patients with stage III disease compared with the initial - I stage. Similar differences were found on stages of the process for TG - they are increased by 1.55 times in stages I to III-d (Table 2).

Table 2. Biochemical parameters of lipid metabolism blood serum of patients with osteoarthritis at different stages of the disease

Index	Norm	I stage OA	II stage OA	III stage OA
Holesterol mmol/l	5,87(4,62;6,55)	6,16(5,04;7,42)	**$6{,}85^{0{,}05}$##(5,33;7,97)**	**$8{,}00^{0{,}01}$#(7,06;9,60)**
Triglycerides mmol/l	0,41(0,14;1,82)	0,88(0,60;1,30)	**$1{,}16^{0{,}05}$##(0,82;1,60)**	**$1{,}73^{0{,}05}$#(1,38;2,00)**
Global lipids g/l	4,56(3,43;8,01)	4,75(3,92;5,83)	5,05(4,29;5,94)	5,20(4,40;7,91)

Note: The superscript - significance level (p) is compared with the norm;

- Statistically significant differences between the 1 and 3-stage disease;

- Statistically significant differences between the 2 and 3-stage disease.

It is known that OA is a disease whose etiology is multifactorial and not fully understood (MinkowitzR.B., 2009). Damage to the articular cartilage of the knee joint surfaces and damage intra-articular structures often have the same clinical picture, which is manifested severe pain. Osteoarthritis dysplastic etiology involves degenerative changes sustavnogohryascha on one or both surfaces of the patella and the femur in the block.

According to our data at OA different etiology significantly increased the concentration of GL synovium, changing the lipid spectrum was equally expressed in the increase in the concentration of HL and decrease in the concentration of triglycerides and there was a redistribution of lipid peroxidation products - significantly increased the concentration of DK primary products. When gonarthrosis traumatic etiology significantly reduced activity of catalase. In other groups not significantly, but reduced the synovium catalase activity, indicating that amplification of oxidative processes in the synovium occurs. Indicators TP development of arthritic process increases as well in

all groups of patients. The concentrations of OMP products happened multiple accumulation of aldehydes. The amount of ketones in all groups were significantly lower than normal. The protein spectrum showed odnotipnyeizmeneniya lead to reduction of α-globulin fractions and increasing β-globulin fraction (Figure 9).

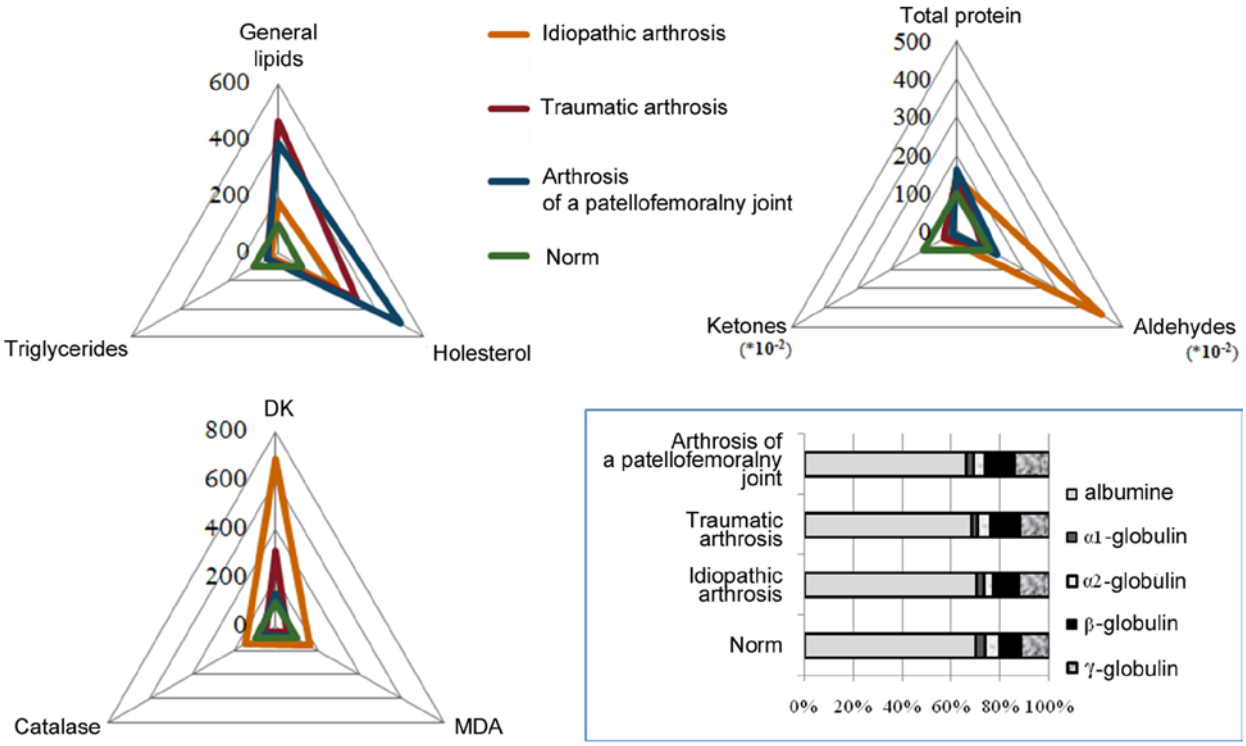


Figure 9. Biochemical parameters of synovial fluid of patients with osteoarthritis of various etiologies (in% of normal)

Thus, a comparative analysis of the results of the research showed that as a result of DDCJ with any etiology (both idiopathic and post-traumatic, dysplastic) in SF is an increase in the concentration of GL and TP. In addition, the range of changes of lipid and protein component of synovial: marked increase in the concentration of HL and decrease in TG concentration redistribution of the concentration of protein fractions with the increase in the proportion of β-globulin and a decrease in α1-and α2-globulin fractions.

At the end point of DDCJ main treatment strategy is joint replacement. After the primary arthroplasty, according to the literature, the favorable results observed in 85% of cases with the analysis horizon of 3 years (KurtzS.,etal.2007; DreesP. etal.2008). Odnakopo as studying long-term results of positive outcomes is significantly reduced, and this reduction is due to the natural duration of the observation period for the operated patients. The group of patients who had identified the instability of the endoprosthesis, was the material from 217 patients from among the patients, operated on for primary knee arthroplasty. This group has been allocated over the term of our experiment (3 years) and presented the observations of 9 217 patients.

Biochemical indicators of SF patients with nestabilnymendoprotezom statistically significantly different from the norm as the values and of the indicators in the group of patients with stable endoprosthesis (Figure 10).

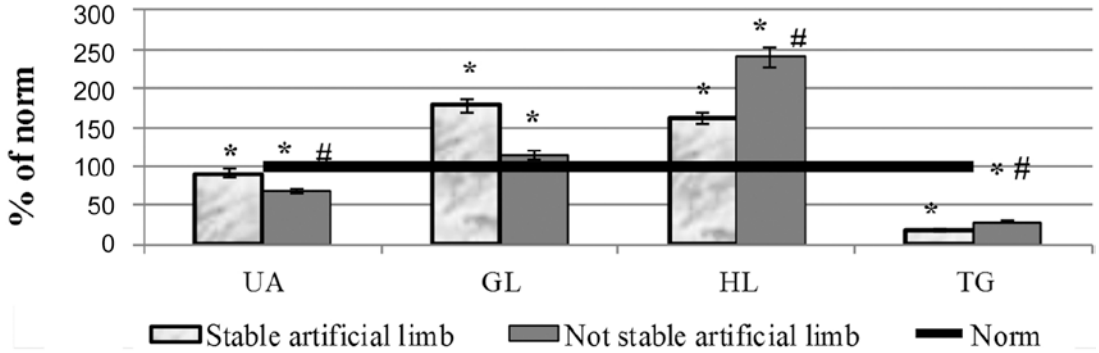
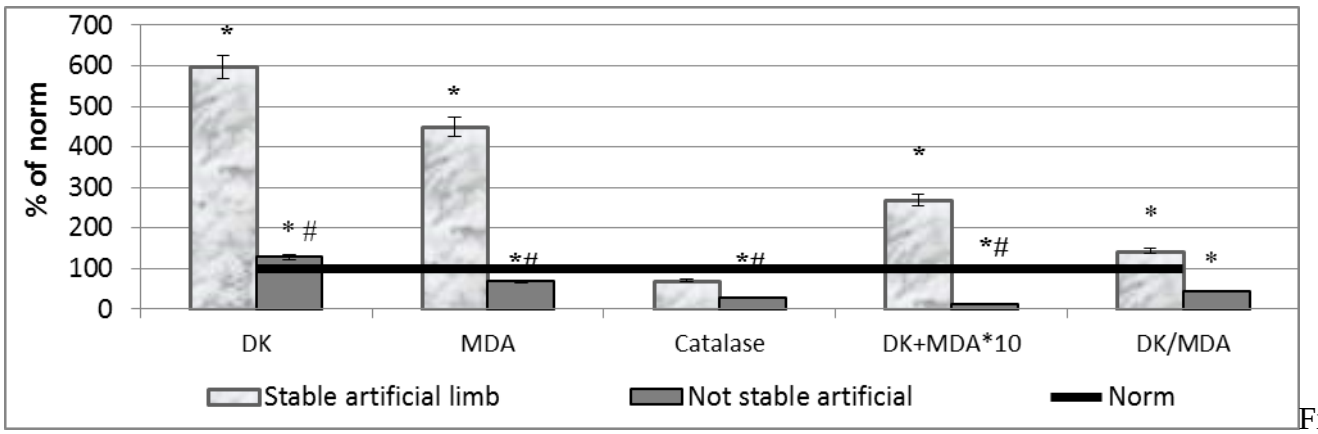


Figure 10. The lipid spectrum of synovial fluid of patients in the group of patients with knee pervichnymendoprotezirovaniem sustavaPrimechanie -. See Fig. 1

In both groups, the concentration of uronic acid is reduced, and reduced by 33% in the endoprosthesis instability is greater than the reference group patients. Concentration GL in patients with unstable endoprosthesis increased with respect to normal values by 2 times; Group differences regarding stability of the endoprosthesis is not statistically significant. Analysis of the lipid spectrum changes showed an increased concentration of HL (at 4 times the rate of more than 2 times - a relatively stable group of prosthesis). TG concentration has been reduced by 3.5 times relative to the norm, but, nevertheless, it is significantly higher than in patients with stable endoprosthesis.Comparative analysis of the results of research concentration peroxidation products at the instability of the endoprosthesis (Figure 11).

When instability of the endoprosthesis levels of lipid peroxidation products decreased sharply compared with a group of stable implant (4.5 times for DK and 6.5 times for the MDA). In comparison with normal values observed increase in the concentration of primary products and a statistically significant reduction in secondary products. Estimated coefficient [DK + MDA] also had differences between the groups of patients. If the group of patients sostabilnymendoprotezom total accumulation of lipid peroxidation was significantly (almost 18 times) higher than the normal values, in the group of patients who subsequently developed instability, this parameter was even below the normal range. In the group with the instability of the endoprosthesis component ratio [DK / MDA] was significantly increased relative to the norm. Analysis of activity of the antioxidant enzyme catalase in the group with stabilnymendoprotezom showed no statistically significant differences from the norm, and in the group with the instability of catalase activity was increased almost 2-fold.



Fi

Fgure 11. The processes of the synovial fluid of patients with lipid peroxidation in patients with knee pervichnymendoprotezirovaniem sustavaPrimechanie -. See Fig. 1

In these patient groups as indicators OMP products and protein spectra were examined (Figure 12).

SF In patients with knee arthroplasty revealing varied concentration of TP, which has consistently increased relative to the norm in groups of stable and unstable prosthesis (from the norm - at 40% of the comparison group - 22%). The concentration of the aldehydes was increased 1.5-fold in the endoprosthesis group stable with respect to normal values, but was two times lower than that of patients in the NE. the comparison group. The concentration of ketones was significantly reduced (by 4 times - from the norm and 1.5 times from Group stable prosthesis). Estimated coefficient [Aldehydes Ketones +] was reduced by 2 times compared with the norm, and 3 times the comparison group. The ratio [Aldehydes / ketones] statistically significantly increased relative to normal values in both study groups (up to 10 times with a stable group, and a 3-fold - in the group with unstable endoprosthesis); while in the group with unstable endoprosthesis this ratio was significantly lower than in group sravneniya.Kontsentratsiya α2-globulin fraction was increased in the group with respect to NE and comparison group had significant differences from the norm

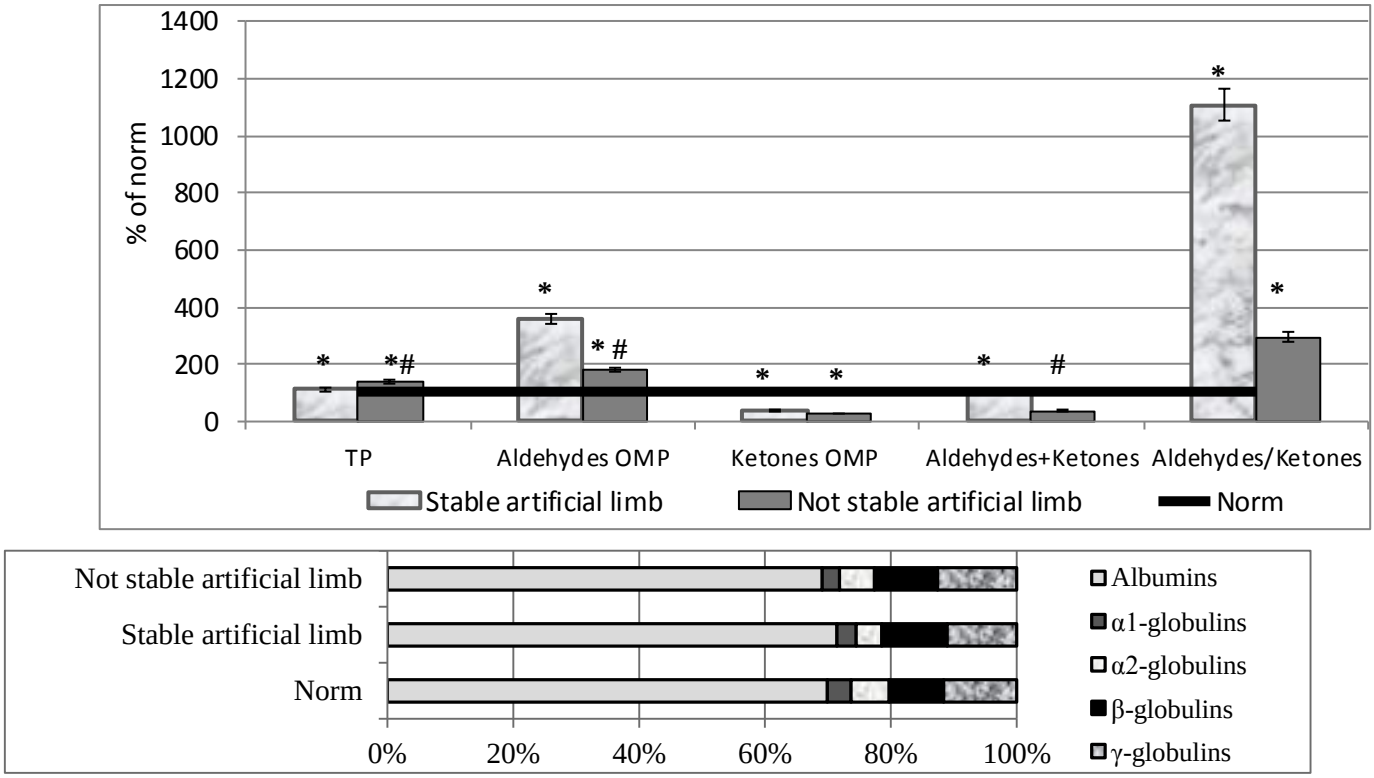


Figure 12. Biochemical parameters of synovial fluid of patients with nestabilnostyuendoproteza as measured by OMB and protein spektraPrimechanie -. See Fig. 1

Thus, highlighting figures with opposite changes in the comparison groups possible criteria in predicting instability during knee arthroplasty defined in SF indicators of lipid peroxidation products - MDA, the total index of lipid peroxidation products [DK + MDA], as well as the activity of the antioxidant enzyme katalazy.Snizhenie indicators peroxidation products with an increase in the activity of key antioxidant enzymes - catalase indicates a risk of developing this complication.

CONCLUSION

Our research has revealed that at different stages of experimental modeling of DDCJ changes prove to be evident in lipid and protein SF spectrum, lipid peroxidation and OMB. The results show that in SF of pathologically changed joints metabolic abnormalities occur earlier than in the serum.

Biochemical parameters of SF peroxidation in patients with OA of various etiology are unidirectional, namely, concentrations of GL and TP, redistribution of lipid peroxidation and OMP products tend to grow.

In addition, the spectrum of the lipid and protein component of synovial changes; the concentration of HL increases, while TG concentration decreases, there is a redistribution of the protein fractions with a decrease in α1-and α2-fraction and an increase in the share of β-globulin. Application of indicators of synovial fluid LPO and OMP together clinical and radiological data provides an opportunity for an objective estimation of the environment in the development of synovial articular pathology, it may also serve as criteria for predicting the instability of the knee joint endoprosthesis.

DEDUCTIONS:

1. Reference values of biochemical parameters of lipid and protein spectrum, lipid peroxidation and oxidative modification of proteins have been determined for the synovial fluid of large human joints. Statistically significant differences have been distinguished in total protein and general al lipid indicators, cholesterol and triglycerides, ketones.
2. In the course of degenerative and dystrophic changes in the joints, the intensity of oxidative modification of proteins in the experimental animals' synovial fluid of tend to decrease, while lipid peroxidation products tend to accumulate: diene conjugates at early stages and diene conjugates and malonic dialdehyde at later stages.
3. In patients with idiopathic OA knee at early as well as at later stages of the disease lipid peroxidation and oxidative modification of proteins prove to intensify. In the synovial fluid the products of their peroxidation accumulate with a predominance of primary products, diene conjugates and aldehydes.
4. In the synovial fluid of patients with idiopathic, post-traumatic and dysplastic osteoarthritis occur unidirectional changes occur in the products of lipid peroxidation and oxidative modification of proteins.
5. Increased activity of catalase and reduced concentration of malonic dialdehyde in the synovial fluid are unfavourable prognostic criteria, indicating the a risk of instability for primary knee arthroplasty.

PRACTICE GUIDELINES

With the introduction of the results of the research into clinical practice, we recommend that it is possible to:

1. Investigation of lipid and protein spectrum, determination of lipid peroxidation and protein should be used to objectively assess the severity of a pathological process in the joint.

2. Using standard indicators of the biochemical composition of the synovial fluid must be carried out taking into account the localization of the pathological process, differentiating performance of knee and elbow.
3. Definition of synovial fluid parameters of lipid peroxidation and catalase activity may bytispolzovany as additional laboratory tests in predicting nestabilnostiendoprotezapri primary knee arthroplasty**.**

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СПИСОК СОКРАЩЕНИЙ

- DDCJ – degenerative-dystrophic changes in the joints
- DK – diene konjugates;
- IA –idiopathic arthritis;
- MDA – malonovij dialdehyde;
- OA -osteoarthritis ;
- TP – total protein;
- GL – global lipids;
- OPM – Oxidative protein modification;
- LPO – lipid peroxidation;

- SF – synovial fluid;
- TG – triglycerides;
- HL – cholesterol.